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Roles of MicroRNA-mediated Drug Resistance in Tumor stem Cells of Small Cell Lung Carcinoma

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14. ABSTRACT

Lung cancer is the leading cause of cancer deaths in the world, and according to the statistics of National Cancer Institute's SEER, 219,440 men and women will be diagnosed and 159,000 patients will die from this disease in 2010. Small cell lung carcinoma (SCLC) is the cause of approximately 20% of lung cancer. Chemo-therapy is the first choice of treatment for this carcinoma because SCLC is often highly malignant and metastasizes to the distant organs even at an early stage. Although SCLC is chemotherapy-sensitive at the initial stage, it becomes ultimately chemo-resistant with worse prognosis. Recent stem cell theory suggests that there is a distinct population of tumor cells that has stem-like characteristics as well as ability of resistance to chemo-therapy. Moreover, several lines of evidence indicated that the self renewal ability of cancer stem cells is regulated by microRNAs. Therefore, **we hypothesize that drug resistance of SCLC is mediated by microRNA in tumor stem cell**. In this project, we are proposing to identify the specific microRNA in the stem cells from SCLC. We also plan to characterize these microRNAs so that we can eventually identify specific target molecules to block the chemo-resistance.

15. SUBJECT TERMS

Prostate cancer, tumor stem cells, dormancy, bone metastasis

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INTRODUCTION

There is no cancer chemo-therapy that is 100% effective against malignant and metastatic tumors because of appearance of recurrent tumor with drug resistance. Resistance to the treatment of anti-cancer drugs is mainly attributed to the heterogeneity of cancer cells. Small cell lung carcinoma (SCLC) is one of the most malignant types of lung cancers, and has median survival of only 2-4 months from diagnosis if patients are not treated (1). This carcinoma is more responsive to chemotherapies compared to the other types of pulmonary carcinomas; however, SCLC readily acquires resistance to the drug treatment. The recent tumor stem cell theory offers an attractive explanation not only for the origin of tumors but also for the chemo-resistance mechanism (2). Moreover, several lines of evidence suggest that drug resistance and self-renewal ability of cancer stem cells are partly controlled by microRNAs (3). Therefore, it is plausible that drug resistance of SCLC is attributed to specific microRNAs in cancer stem cells.

BODY

Aim 1. To identify specific microRNAs in tumor stem cell from SCLC.

(a) We will isolate tumor stem cells from primary and recurrent tumors from SCLC patients and perform microRNA array analysis.
(b) We will transfect a lentivirus-based microRNA expression library to the isolated tumor stem cells of SCLC and transplant them to NOD/SCID mice followed by treatment with cisplatin. After withdrawal of the drug, recurrent tumor will be removed, and stem cells will be isolated and microRNAs in the stem cells will be identified.

Progress

For this purpose, we first use two SCLC cell lines, namely HTB119 and HTB173. HTB119 was isolated from primary tumor, while HTB173 was isolated from bone metastatic site and it grows as suspension. They were cultured in RPI1640 medium and the cells were harvested for stem cell isolation. We used CD133 or ALDH for sorting cancer stem cells and found that the CD133+ population showed significantly higher tumor initiating ability compared to ALDH-sorted cells. Currently, we are testing whether CD133+/ALDH+ cells represent better stemness. We also found that CD133+ cells were more capable of generating spheres in serum-free culture compared to the ALDH+ cells. We then extracted RNAs from these cells and they were subjected to the microRNA array analysis. The results of our analysis indicate that mir16 and 21 are significantly upregulated in HTB173 which is a metastatic stem cell. We have confirmed these results by TaqMan qRT-PCR using the original stem cell populations in two cell lines. The results of Targetscan-based analysis indicate that mir16 is capable of blocking BCL2 (anti-apoptotic gene), while mir21 targets PTEN and Jag1 that are play important roles in stem cell physiology. We are confirming this hypothesis by knocking down the microRNA in stem cell and examine the expression of the target genes.
The next step is to identify microRNAs in CSC of SCLC in patients. We have a contract with Conversant Biologic to harvest such tissues. However, these cells are scarce and we have to wait for a few more months. These samples will be examined for the expression of miR16 and 21. They will also be examined by the global profiling for microRNA expression.

For the second subaim, we have cultured HTB119 and HTB173, and they were infected with the lentivirus-base microRNA expression library. These cells were then selected for puromycine resistance to establish the cell lines. Early generations of these cells were secured and stocked as frozen culture. We have isolated the stem cell population from these cell lines by sorting with CD133 or ALDH marker and found that these populations were indeed capable of generating spheres in vitro,

a hallmark of stemness. Therefore, we are planning to transplant these cells into NOD/SCID mice followed by treating them with Cisplatin so that we can isolate stem cells from these tumors.

Aim 2. To verify the function of the identified microRNAs in tumor stem cells *in vitro* and *in vivo*. We will examine whether the identified microRNAs indeed cause cisplatin-resistance by ectopically expressing the RNA in SCLC stem cell followed by testing their chemo-resistance *in vitro* and *in vivo*.

Progress
This experiment is dependent on the results of Specific aim 1(b). Therefore, we are waiting for the results as we planned. We expect that the result will be obtained in the next 3 months.

KEY RESEARCH ACCOMPLISHMENTS

1. We have successfully isolated CSCs from small cell lung carcinoma and found that they have strong tumor-initiating capability.
2. The result of global expression analysis of these stem cells revealed that mir16 and 21 are significantly up-regulated in the CSCs from an aggressive form of SCLC.
3. We have established a tissue cell library that expresses microRNAs in cancer stem cells of SCLC.

REPORTABLE OUTCOMES

Peer reviewed publications
None.

Employment
1. Kerui Wu (Graduate student) has been partly supported by the current grant.

CONCLUSIONS

We have established a method of isolating cancer stem cells from human SCLC. They are highly tumorigenic in animal and generate sphere formation *in vitro*. The highly metastatic stem cells express significantly higher amount of mir16 and mir21 that have been characterized as oncomirs. We also established cell lines expressing microRNA library and we are planning for *in vivo* screening for microRNAs that are involved in drug resistance of SCLC. By combining the results of these two experiments, we should be able to identify candidate microRNAs that are involved in chemoresistance of CSCs in SCLC.

So what?
Although combined modality therapies have shown significant improvement of long-term survival of SCLC patients, the overall survival rate of this cancer is still much lower than other types of lung cancers. To overcome this limitation, development of a more effective drug for the treatment of this carcinoma is urgently needed. We believe that the results of our project which is uniquely focused on microRNAs in tumor stem cells of chemo-resistant SCLC will shed new light on the drug-resistance mechanism and open a possibility of developing a novel therapeutic drug for a better treatment of SCLC.

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